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Patents ADP number *(if you know it)* —

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RECEPTORS

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# RECEPTORS

The present invention relates to phage particles displaying T cell receptors (TCRs).

## Background to the Invention

*Native TCRs*

As is described in, for example, WO 99/60120 TCRs mediate the recognition of specific Major Histocompatibility Complex (MHC)-peptide complexes by T cells and, as such, are essential to the functioning of the cellular arm of the immune system.

Antibodies and TCRs are the only two types of molecules which recognise antigens in a specific manner, and thus the TCR is the only receptor for particular peptide antigens presented in MHC, the alien peptide often being the only sign of an abnormality within a cell. T cell recognition occurs when a T-cell and an antigen presenting cell (APC) are in direct physical contact, and is initiated by ligation of antigen-specific TCRs with pMHC complexes.

The native TCR is a heterodimeric cell surface protein of the immunoglobulin superfamily which is associated with invariant proteins of the CD3 complex involved in mediating signal transduction. TCRs exist in αβ and γδ forms, which are structurally similar but have quite distinct anatomical locations and probably functions. The MHC class I and class II ligands are also immunoglobulin superfamily proteins but are specialised for antigen presentation, with a highly polymorphic peptide binding site which enables them to present a diverse array of short peptide fragments at the APC cell surface.

The extracellular portion of native heterodimeric αβ and γδ TCRs consist of two polypeptides each of which has a membrane-proximal constant domain, and a membrane-distal variable domain. Each of the constant and variable domains includes an intra-chain disulfide bond. The variable domains contain the highly polymorphic loops analogous to the complementarity determining regions (CDRs) of antibodies.

CDR3 of αβ TCRs interact with the peptide presented by MHC, and CDRs 1 and 2 of αβ TCRs interact with the peptide and the MHC. The diversity of TCR sequences is generated via somatic rearrangement of linked variable (V), diversity (D), joining (J), and constant genes

Functional α and γ chain polypeptides are formed by rearranged V-J-C regions, whereas β and δ chains consist of V-D-J-C regions. The extracellular constant domain has a membrane proximal region and an immunoglobulin region. There are single α and δ chain constant domains, known as TRAC and TRDC respectively. The β chain constant domain is composed of one of two different β constant domains, known as TRBC1 and TRBC2 (IMGT nomenclature). There are four amino acid changes between these β constant domains, three of which are within the domains used to produce the single-chain TCRs displayed on phage particles of the present invention. These changes are all within exon 1 of TRBC1 and TRBC2: $N_4K_5$->$K_4N_5$ and $F_{37}$->Y (IMGT numbering, differences TRBC1->TRBC2), the final amino acid change between the two TCR β chain constant regions being in exon 3 of TRBC1 and TRBC2: $V_1$->E. The constant γ domain is composed of one of either TRGC1, TRGC2(2x) or TRGC2(3x). The two TRGC2 constant domains differ only in the number of copies of the amino acids encoded by exon 2 of this gene that are present.

The extent of each of the TCR extracellular domains is somewhat variable. However, a person skilled in the art can readily determine the position of the domain boundaries using a reference such as The T Cell Receptor Facts Book, Lefranc & Lefranc, Publ. Academic Press 2001.

*Recombinant TCRs*

The production of recombinant TCRs is beneficial as these provide soluble TCR analogues suitable for the following purposes:

- Studying the TCR / ligand interactions (e.g. pMHC for αβ TCRs)

- Screening for inhibitors of TCR-associated interactions
- Providing the basis for potential therapeutics

A number of constructs have been devised to date for the production of recombinant TCRs. These constructs fall into two broad classes, single-chain TCRs and dimeric TCRs, the literature relevant to these constructs is summarised below.

Single-chain TCRs (scTCRs) are artificial constructs consisting of a single amino acid strand, which like native heterodimeric TCRs bind to MHC-peptide complexes. Unfortunately, attempts to produce functional alpha/beta analogue scTCRs by simply linking the alpha and beta chains such that both are expressed in a single open reading frame have been unsuccessful, presumably because of the natural instability of the alpha-beta soluble domain pairing.

Accordingly, special techniques using various truncations of either or both of the alpha and beta chains have been necessary for the production of scTCRs. These formats appear to be applicable only to a very limited range of scTCR sequences. Soo Hoo *et al* (1992) PNAS. **89** (10): 4759-63 report the expression of a mouse TCR in single chain format from the 2C T cell clone using a truncated beta and alpha chain linked with a 25 amino acid linker and bacterial periplasmic expression (see also Schodin *et al* (1996) Mol. Immunol. **33** (**9**): 819-29). This design also forms the basis of the m6 single-chain TCR reported by Holler *et al* (2000) PNAS. **97** (10): 5387-92 which is derived from the 2C scTCR and binds to the same H2-Ld-restricted alloepitope. Shusta *et al* (2000) Nature Biotechnology **18**: 754-759 report using single-chain 2 C TCR constructs in yeast display experiments, which produced mutated TCRs with, enhanced thermal stability and solubility. This report also demonstrated the ability of these displayed 2C TCRs to selectively bind cells expressing their cognate pMHC. Khandekar *et al* (1997) J. Biol. Chem. **272** (51): 32190-7 report a similar design for the murine D10 TCR, although this scTCR was fused to MBP and expressed in bacterial cytoplasm (see also Hare *et al* (1999) Nat. Struct. Biol. **6** (6): 574-81). Hilyard *et al* (1994) PNAS. **91** (19): 9057-61 report a

human scTCR specific for influenza matrix protein-HLA-A2, using a Vα-linker-Vβ design and expressed in bacterial periplasm.

Chung *et al* (1994) PNAS. **91** (26) 12654-8 report the production of a human scTCR using a Vα-linker-Vβ-Cβ design and expression on the surface of a mammalian cell line. This report does not include any reference to peptide-HLA specific binding of the scTCR. Plaksin *et al* (1997) J. Immunol. **158** (5): 2218-27 report a similar Vα-linker-Vβ-Cβ design for producing a murine scTCR specific for an HIV gp120-H-2D$^{d}$ epitope. This scTCR is expressed as bacterial inclusion bodies and refolded *in vitro*.

A number of papers describe the production of TCR heterodimers which include the native disulphide bridge which connects the respective subunits (Garboczi, *et al.*, (1996), Nature **384**(6605): 134-41; Garboczi, *et al.*, (1996), J Immunol **157**(12): 5403-10; Chang *et al.*, (1994), PNAS USA **91**: 11408-11412; Davodeau *et al.*, (1993), *J. Biol. Chem.* **268**(21): 15455-15460; Golden *et al.*, (1997), J. Imm. Meth. **206**: 163-169; US Patent No. 6080840). However, although such TCRs can be recognised by TCR-specific antibodies, none were shown to recognise its native ligand at anything other than relatively high concentrations and/or were not stable.

In WO 99/60120, a soluble TCR is described which is correctly folded so that it is capable of recognising its native ligand, is stable over a period of time, and can be produced in reasonable quantities. This TCR comprises a TCR α or γ chain extracellular domain dimerised to a TCR β or δ chain extracellular domain respectively, by means of a pair of C-terminal dimerisation peptides, such as leucine zippers. This strategy of producing TCRs is generally applicable to all TCRs.

Reiter *et al*, Immunity, 1995, **2**:281-287, details the construction of a soluble molecule comprising disulphide-stabilised TCR α and β variable domains, one of which is linked to a truncated form of Pseudomonas exotoxin (PE38). One of the stated reasons for producing this molecule was to overcome the inherent instability of single-

chain TCRs. The position of the novel disulphide bond in the TCR variable domains was identified via homology with the variable domains of antibodies, into which these have previously been introduced (for example see Brinkmann, *et al.* (1993), Proc. Natl. Acad. Sci. USA **90:** 7538-7542, and Reiter, *et al.* (1994) Biochemistry **33:** 5451-5459). However, as there is no such homology between antibody and TCR constant domains, such a technique could not be employed to identify appropriate sites for new inter-chain disulphide bonds between TCR constant domains.

As mentioned above Shusta *et al* (2000) Nature Biotechnology **18**: 754-759 report using single-chain 2 C TCR constructs in yeast display experiments. The principle of displaying scTCRs on phage particles has previously been discussed. For example, WO 99/19129 details the production of scTCRs, and summarise a potential method for the production of phage particles displaying scTCRs of the Vα-Linker-Vβ Cβ format. However, this application contains no exemplification demonstrating the production of said phage particles displaying TCR. The application does however refer to a co-pending application:

"The construction of DNA vectors including a DNA segment encoding a sc-TCR molecules fused to a bacteriophage coat protein (gene II or gene VIII) have been described in said pending U.S. application No. 08/813,781."

Furthermore, this application relies on the ability of anti-TCR antibodies or super-antigen MHC complexes to recognise the soluble, non-phage displayed, scTCRs produced to verify their correct conformation. Therefore, true peptide-MHC binding specificity of the scTCRs, in any format, is not conclusively demonstrated.

*Screening Use*

A number of important cellular interactions and cell responses, including the TCR-mediated immune synapse, are controlled by contacts made between cell surface receptors and ligands presented on the surfaces of other cells. These types of specific molecular contacts are of crucial importance to the correct biochemical regulation in

the human body and are therefore being studied intensely. In many cases, the objective of such studies is to devise a means of modulating cellular responses in order to prevent or combat disease.

Therefore, methods with which to identify compounds that bind with some degree of specificity to human receptor or ligand molecules are important as leads for the discovery and development of new disease therapeutics. In particular, compounds that interfere with certain receptor-ligand interactions have immediate potential as therapeutic agents or carriers.

Advances in combinatorial chemistry, enabling relatively easy and cost-efficient production of very large compound libraries have increased the scope for compound testing enormously. Now the limitations of screening programmes most often reside in the nature of the assays that can be employed, the production of suitable receptor and ligand molecules and how well these assays can be adapted to high throughput screening methods.

*Phage Display Methods*

The phage display technique is based on the ability of bacteriophage particles to express a heterologous peptide or polypeptide fused to their surface proteins. (Smith (1985) Science **217** 1315-1317). The procedure is quite general, and well understood in the art for the display of polypeptide monomers. However, in the case of ~~polypeptides which in their native form associate as dimers, the phage display process~~ appears less well discussed in the literature. For monomeric polypeptide display there are two main procedures:

Firstly (Method A) by inserting DNA encoding for the heterologous peptide or polypeptide into a vector (phagemid) fused to the DNA encoding for a bacteriophage coat protein. The expression of phage particles displaying the heterologous peptide or polypeptide is then carried out by transfecting bacterial cells with the phagemid, and then infecting the transformed cells with a 'helper phage'. The helper phage acts as a

source of the phage proteins not encoded by the phagemid required to produce a functional phage particle.

Secondly (Method B), by inserting DNA encoding the heterologous peptide or polypeptide into a complete phage genome fused to the DNA encoding a bacteriophage coat protein. The expression of phage particles displaying the heterologous peptide or polypeptide is then carried out by infecting bacterial cells with the phage genome. This method has the advantage of the first method of being a 'single-step' process. However, the size of the heterologous DNA sequence that can be successfully packaged into the resuting phage particles is reduced. M13, T7 and Lambda are examples of suitable phages for this method.

A variation on (Method B) the involves adding a DNA sequence encoding a nucleotide binding domain to the DNA in the phage genome encoding the heterologous peptide be displayed, and further adding the corresponding nucleotide binding site to the phage genome. This causes the heterologous peptide to become directly attached to the phage genome. This peptide/genome complex is then packaged into a phage particle which displays the heterologous peptide. This method is fully described in WO 99/11785.

The phage particles can then be recovered and used to study the binding characteristics of the heterologous peptide or polypeptide. Once isolated, phagemid or phage DNA can be recovered from the TCR-displaying phage particle, and this DNA can be replicated via PCR. The PCR product can be used to sequence the heterologous peptide or polypeptide displayed by a given phage particle.

The phage display of single-chain antibodies and fragments thereof, has become a routine means of studying the binding characteristics of these polypeptides. There are numerous books available that review phage display techniques and the biology of the bacteriophage. (See, for example, Phage Display – A Laboratory Manual, Barbas *et al.*, (2001) Cold Spring Harbour Laboratory Press).

A third phage display method (Method C) relies on the fact that heterologous polypeptides having a cysteine residue at a desired location can be expressed in a soluble form by a phagemid or phage genome, and caused to associate with a modified phage surface protein also having a cysteine residue at a surface exposed position, via the formation of a disulphide linkage between the two cysteines. WO 01/ 05950 details the use of this alternative linkage method for the expression of single-chain antibody-derived peptides.

**Brief Description of the Invention**

This invention makes available phage particles displaying alpha/beta and gamma/delta-analogue scTCR and dTCR constructs, the former having an intrachain disulfide bond and the latter preferably having an interchain disulfide bond, said bonds being between regions of the molecule corresponding to the constant regions of native TCRs and contributing to the stability of the molecule. The presence of these disulfide bonds assists the displayed TCRs to fold into a conformation such that the variable region sequences of the dTCR polypeptide pair or scTCR polypeptide are mutually orientated substantially as in native TCRs. Such phage-displayed TCRs are useful for screening purposes.

**Detailed Description of the Invention**

The present invention provides a phage particle displaying on its surface a single chain ~~T cell receptor (scTCR) polypeptide or dimeric TCR (dTCR) polypepide pair. Said~~ scTCR polypeptide, or dTCR polypeptide pair are constituted by TCR amino acid sequences corresponding to TCR extracellular constant and variable region sequences, with a variable region sequence of the scTCR corresponding to a variable region sequence of one TCR chain being linked by a linker sequence to a constant region sequence corresponding to a constant region sequence of another TCR chain; the variable region sequences of the dTCR polypeptide pair or scTCR polypeptide are mutually orientated substantially as in native TCRs; and in the case of the scTCR

polypeptide a disulfide bond which has no equivalent in native T cell receptors links residues of the polypeptide.

In the scTCRs or dTCRs of the invention the requirement that the variable region sequences are mutually orientated substantially as in native αβ T cell receptors is tested by confirming that the phage displayed TCR binds to its cognate peptide-MHC complex - if binding occurs, then the requirement is met. In the case of γδ TCRs of the present invention the cognate ligands for these molecules are unknown therefore secondary means of verifying their conformation such as recognition by antibodies can be employed. The monoclonal antibody MCA991T (available from Serotec), specific for the δ chain variable region, is an example of an antibody appropriate for this task.

The scTCRs or dTCRs of the present invention may be displayed on phage particles by, for example, the following two means:

The C-terminus of one member of the dTCR polypeptide pair, or the C-terminus of the scTCR polypeptide, can be directly linked by a peptide bond to a surface exposed residue of the phage. The said surface exposed residue is preferable at the N-terminus of the gene product of bacteriophage gene III or gene VIII.; and

The C-terminus of one member of the dTCR polypeptide pair, or the C-terminus of the scTCR polypeptide, is linked by a disulfide bond to a surface exposed cysteine residue of the phage via an introduced cysteine residue. The said surface exposed residue is again preferable at the N-terminus of the gene product of bacteriophage gene III or gene VIII.

*The displayed scTCR*

The displayed scTCR polypeptide may be, for example, one which has

a first segment constituted by an amino acid sequence corresponding to a TCR α or δ chain variable region sequence fused to the N terminus of an amino acid sequence corresponding to a TCR α chain constant region extracellular sequence,

a second segment constituted by an amino acid sequence corresponding to a TCR β or γ chain variable region fused to the N terminus of an amino acid sequence corresponding to TCR β chain constant region extracellular sequence,

a linker sequence linking the C terminus of the first segment to the N terminus of the second segment, or vice versa, and

a disulfide bond between the first and second chains, said disulfide bond being one which has no equivalent in native αβ or γδ T cell receptors,

the length of the linker sequence and the position of the disulfide bond being such that the variable region sequences of the first and second segments are mutually orientated substantially as in native αβ or γδ T cell receptors.

*The displayed dTCR*

The dTCR which is displayed on the phage particle may be one which is constituted by

a first polypeptide wherein a sequence corresponding to a TCR α or δ chain variable region sequence is fused to the N terminus of a sequence corresponding to a TCR α chain constant region extracellular sequence, and

a second polypeptide wherein a sequence corresponding to a TCR β or γ chain variable region sequence fused to the N terminus a sequence corresponding to a TCR β chain constant-region-extracellular-sequence,

the first and second polypeptides being linked by a disulfide bond which has no equivalent in native αβ or γδ T cell receptors.

*dTCR Polypeptide Pair and scTCR Polypeptide*

The constant region extracellular sequences present in the scTCRs or dTCRs preferably correspond to those of a human TCR, as do the variable region sequences. However, the correspondence between such sequences need not be 1:1 on an amino acid level. N- or C-truncation, and/or amino acid deletion and/or substitution relative to the corresponding human TCR sequences is acceptable, provided the overall result is mutual orientation of the α and β variable region sequences, or γ and δ variable region sequences is as in native αβ, or γδ T cell receptors respectively. In particular, because the constant region extracellular sequences present in the first and second segments are not directly involved in contacts with the ligand to which the scTCR or dTCR binds, they may be shorter than, or may contain substitutions or deletions relative to, extracellular constant domain sequences of native TCRs.

The constant region extracellular sequence present in one of the dTCR polypeptide pair, or in the first segment of a scTCR polypeptide may include a sequence corresponding to the extracellular constant Ig domain of a TCR α chain, and/or the constant region extracellular sequence present in the other member of the pair or second segment may include a sequence corresponding to the extracellular constant Ig domain of a TCR β chain.

In one embodiment of the invention, one member of the polypeptide pair or the first segment of the scTCR polypeptide corresponds to substantially all the variable region of a TCR α chain fused to the N terminus of substantially all the extracellular domain of the constant region of an TCR α chain; and/or the other member of the pair or second segment corresponds to substantially all the variable region of a TCR β chain fused to the N terminus of substantially all the extracellular domain of the constant region of a TCR β chain.

In another embodiment, the constant region extracellular sequences present in the dTCR polypeptide pair or first and second segments of the scTCR polypeptide correspond to the constant regions of the α and β chains of a native TCR truncated at their C termini such that the cysteine residues which form the native inter-chain disulfide bond of the TCR are excluded. Alternatively those cysteine residues may be substituted by another amino acid residue such as serine or alanine, so that the native disulfide bond is deleted. In addition, the native TCR β chain contains an unpaired cysteine residue and that residue may be deleted from, or replaced by a non-cysteine residue in, the β sequence of the scTCR of the invention.

In one particular embodiment of the invention, the TCR α and β chain variable region sequences present in the dTCR polypeptide pair or first and second segments of the scTCR polypeptide may together correspond to the functional variable domain of a first TCR, and the TCR α and β chain constant region extracellular sequences present in the dTCR polypeptide pair or first and second segments of the scTCR polypeptide may correspond to those of a second TCR, the first and second TCRs being from the same species. Thus the α and β chain variable region sequences present in dTCR polypeptide pair or first and second segments of the scTCR polypeptide may correspond to those of a first human TCR, and the α and β chain constant region extracellular sequences may correspond to those of a second human TCR. For example, A6 Tax sTCR constant region extracellular sequences can be used as a framework onto which heterologous α and β variable domains can be fused.

In another embodiment of the invention, the TCR δ and γ chain variable region sequences present in the dTCR polypeptide pair or first and second segments of the scTCR polypeptide respectively, may together correspond to the functional variable domain of a first TCR, and the TCR α and β chain constant region extracellular sequences present in the dTCR polypeptide pair or first and second segments of the scTCR polypeptide respectively, may correspond to those of a second TCR, the first

and second TCRs being from the same species. Thus the δ and γ chain variable region sequences present in the dTCR polypeptide pair or first and second segments of the scTCR polypeptide may correspond to those of a first human TCR, and the α and β chain constant region extracellular sequences may correspond to those of a second human TCR. For example, A6 Tax sTCR constant region extracellular sequences can be used as a framework onto which heterologous γ and δ variable domains can be fused.

In one particular embodiment of the invention, the TCR α and β, or δ and γ chain variable region sequences present in the dTCR polypeptide pair or first and second segments of the scTCR polypeptide may together correspond to the functional variable domain of a first human TCR, and the TCR α and β chain constant region extracellular sequences present in the dTCR polypeptide pair or first and second segments of the scTCR polypeptide may correspond to those of a second non-human TCR, Thus the α and β, or δ and γ chain variable region sequences present dTCR polypeptide pair or first and second segments of the scTCR polypeptide may correspond to those of a first human TCR, and the α and β chain constant region extracellular sequences may correspond to those of a second non-human TCR. For example, murine TCR constant region extracellular sequences can be used as a framework onto which heterologous human α and β TCR variable domains can be fused.

*Linker in the scTCR Polypeptide*

For scTCR-displaying phage particles of the present invention, a linker sequence links the first and second TCR segments, to form a single polypeptide strand. The linker sequence may, for example, have the formula -P-AA-P- wherein P is proline and AA represents an amino acid sequence wherein the amino acids are glycine and serine.

For the scTCR displayed by phage particles of the present invention to bind to a ligand, MHC-peptide complex in the case of αβ TCRs, the first and second segments must be paired so that the variable region sequences thereof are orientated for such

binding. Hence the linker should have sufficient length to span the distance between the C terminus of the first segment and the N terminus of the second segment, or vice versa. On the other hand excessive linker length should preferably be avoided, in case the end of the linker at the N-terminal variable region sequence blocks or reduces bonding of the scTCR to the target ligand.

For example, in the case where the constant region extracellular sequences present in the first and second segments correspond to the constant regions of the α and β chains of a native TCR truncated at their C termini such that the cysteine residues which form the native interchain disulfide bond of the TCR are excluded, and the linker sequence links the C terminus of the first segment to the N terminus of the second segment, the linker may consist of from 26 to 41, for example 29, 30, 31 or 32 amino acids, and a particular linker has the formula -PGGG-$(SGGGG)_5$-P- wherein P is proline, G is glycine and S is serine.

*Inter-chain Disulfide bond*

A principle characterising feature of the scTCRs displayed by phage particles of the present invention, and preferably a feature of the displayed dTCRs, is ~~the~~ a disulfide bond between the constant region extracellular sequences of the dTCR polypeptide pair or first and second segments of the scTCR polypeptide. That bond may correspond to the native inter-chain disulfide bond present in native dimeric αβ TCRs, or may have no counterpart in native TCRs, being between cysteines specifically incorporated into the constant region extracellular sequences of dTCR polypeptide pair or first and second segments of the scTCR polypeptide. In some cases, both a native and a non-native disulfide bond may be desirable.

The position of the disulfide bond is subject to the requirement that the variable region sequences of dTCR polypeptide pair or first and second segments of the scTCR polypeptide are mutually orientated substantially as in native αβ or γδ T cell receptors.

The disulfide bond may be formed by mutating non-cysteine residues on the first and second segments to cysteine, and causing the bond to be formed between the mutated residues. Residues whose respective β carbons are approximately 6 Å (0.6 nm) or less, and preferably in the range 3.5 Å (0.35 nm) to 5.9 Å (0.59 nm) apart in the native TCR are preferred, such that a disulfide bond can be formed between cysteine residues introduced in place of the native residues. It is preferred if the disulfide bond is between residues in the constant immunoglobulin region, although it could be between residues of the membrane proximal region. Preferred sites where cysteines can be introduced to form the disulfide bond are the following residues in exon 1 of TRAC*01 for the TCR α chain and TRBC1*01 or TRBC2*01 for the TCR β chain:

| TCR α chain | TCR β chain | Native β carbon separation (nm) |
|---|---|---|
| Thr 48 | Ser 57 | 0.473 |
| Thr 45 | Ser 77 | 0.533 |
| Tyr 10 | Ser 17 | 0.359 |
| Thr 45 | Asp 59 | 0.560 |
| Ser 15 | Glu 15 | 0.59 |

Now that the residues in human TCRs which can be mutated into cysteine residues to form a new interchain disulfide bond in dTCRs or scTCRs displayed according to the invention have been identified, those of skill in the art will be able to mutate TCRs of other species in the same way to produce a dTCR or scTCR of that species for pahge display. In humans, the skilled person merely needs to look for the following motifs in the respective TCR chains to identify the residue to be mutated (the shaded residue is the residue for mutation to a cysteine).

α Chain Thr 48: DSDVYITDK[T]VLDMRSMDFK (amino acids 39-58 of exon 1 of the TRAC*01 gene)

α Chain Thr 45: QSKDSDVYI[T]DKTVLDMRSM(amino acids 36-55 of exon 1 of the TRAC*01 gene)

α Chain Tyr 10: DIQNPDPAV[Y]QLRDSKSSDK(amino acids 1-20 of exon 1 of the TRAC*01 gene)

α Chain Ser 15: DPAVYQLRD[S]KSSDKSVCLF(amino acids 6-25 of exon 1 of the TRAC*01 gene)

β Chain Ser 57: NGKEVHSGV[S]TDPQPLKEQP(amino acids 48- 67 of exon 1 of the TRBC1*01 & TRBC2*01 genes)

β Chain Ser 77: ALNDSRYAL[S]SRLRVSATFW(amino acids 68- 87 of exon 1 of the TRBC1*01 & TRBC2*01 genes)

β Chain Ser 17: PPEVAVFEP[S]EAEISHTQKA(amino acids 8- 27 of exon 1 of the TRBC1*01 & TRBC2*01 genes)

β Chain Asp 59: KEVHSGVST[D]PQPLKEQPAL(amino acids 50- 69 of exon 1 of the TRBC1*01 & TRBC2*01 genes gene)

β Chain Glu 15: VFPPEVAVF[E]PSEAEISHTQ(amino acids 6- 25 of exon 1 of the TRBC1*01 & TRBC2*01 genes)

In other species, the TCR chains may not have a region which has 100% identity to the above motifs. However, those of skill in the art will be able to use the above motifs to identify the equivalent part of the TCR α or β chain and hence the residue to be mutated to cysteine. Alignment techniques may be used in this respect. For example, ClustalW, available on the European Bioinformatics Institute website (http://www.ebi.ac.uk/index.html) can be used to compare the motifs above to a particular TCR chain sequence in order to locate the relevant part of the TCR sequence for mutation.

The present invention includes within its scope phage displayed αβ and γδ-analogue scTCRs, as well as those of other mammals, including, but not limited to, mouse, rat, pig, goat and sheep. As mentioned above, those of skill in the art will be able to determine sites equivalent to the above-described human sites at which cysteine residues can be introduced to form an inter-chain disulfide bond. For example, the following shows the amino acid sequences of the mouse Cα and Cβ soluble domains, together with motifs showing the murine residues equivalent to the human residues mentioned above that can be mutated to cysteines to form a TCR interchain disulfide bond (where the relevant residues are shaded):

Mouse Cα soluble domain:

PYIQNPEPAVYQLKDPRSQDSTLCLFTDFDSQINVPKTMESGTFITDKTVLDMK
AMDSKSNGAIAWSNQTSFTCQDIFKETNATYPSSDVP

Mouse Cβ soluble domain:

EDLRNVTPPKVSLFEPSKAEIANKQKATLVCLARGFFPDHVELSWWVNGREV
HSGVSTDPQAYKESNYSYCLSSRLRVSATFWHNPRNHFRCQVQFHGLSEEDK
WPEGSPKPVTQNISAEAWGRAD

Murine equivalent of human α Chain Thr 48: ESGTFITDK[T]VLDMKAMDSK

Murine equivalent of human α Chain Thr 45: KTMESGTFI[T]DKTVLDMKAM

Murine equivalent of human α Chain Tyr 10: YIQNPEPAV[Y]QLKDPRSQDS

Murine equivalent of human α Chain Ser 15: AVYQLKDPR[S]QDSTLCLFTD

Murine equivalent of human β Chain Ser 57: NGREVHSGV[S]TDPQAYKESN

Murine equivalent of human β Chain Ser 77: KESNYSYCL[S]SRLRVSATFW

Murine equivalent of human β Chain Ser 17: PPKVSLFEPSKAEIANKQKA

Murine equivalent of human β Chain Asp 59: REVHSGVSTDPQAYKESNYS

Murine equivalent of human β Chain Glu 15: VTPPKVSLFEPSKAEIANKQ

As discussed above, the A6 Tax sTCR extracellular constant regions can be used as framework onto which heterologous variable domains can be fused. It is preferred that the heterologous variable region sequences are linked to the constant region sequences at any point between the disulfide bond and the N termini of the constant region sequences. In the case of the A6 Tax TCR α and β constant region sequences, the disulfide bond may be formed between cysteine residues introduced at amino acid residues 158 and 172 respectively. Therefore it is preferred if the heterologous α and β chain variable region sequence attachment points are between residues 159 or 173 and the N terminus of the α or β constant region sequences respectively.

*Phage Display.*

For scTCR phage display, the scTCR polypeptide is expressed according to any of the three general prior art techniques discussed earlier as Methods A, B, and C. For the scTCRs displayed by phage particles of the present invention to bind to a ligand, MHC-peptide complex in the case of αβ TCRs, the first and second segments must be paired so that the variable region sequences thereof are orientated for such binding. This correct pairing is assisted by the introduction of a disulfide bond in the extracellular constant region of the scTCR. Without wanting to be limited by theory, the novel disulfide bond is believed to provide extra stability to the scTCR during the folding process and thereby facilitating correct pairing of the first and second segments.

For dTCR phage display, one of the dTCR polypeptide pair is expressed according to any of the three general prior art techniques discussed earlier as Methods A, B, and C as if it were eventually to be displayed as a monomeric polypeptide on the phage, and the other of the dTCR polypeptide pair is co-expressed in the same host cell. As the phage particle self assembles, the two polypeptides self associate for display as a dimer on the phage. Again, in the preferred embodiment of this aspect of the invention, correct folding during association of the polypeptide pair is assisted by a disulfide bond between the constant sequences, as discussed above. Further details of a procedure for phage display of a dTCR having an interchain disulfide bond appear in the Examples herein.

*Additional Aspects*

A phage particle displaying a scTCR or dTCR (which preferably is constituted by constant and variable sequences corresponding to human sequences) of the present invention may be provided in substantially pure form, or as a purified or isolated preparation. For example, it may be provided in a form which is substantially free of other proteins.

A phage particle displaying a plurality of scTCRs or dTCRs of the present invention may be provided in a multivalent complex. Thus, the present invention provides, in one aspect, a multivalent T cell receptor (TCR) complex, which comprises a phage particle displaying a plurality of scTCRs or dTCRs as described herein. Each of the plurality of said scTCRs or dTCRs is preferably identical.

In a further aspect, the invention provides a method for detecting TCR ligand complexes, which comprises:

a. providing a TCR-displaying phage of the current invention
b. contacting the TCR-displaying phage with the putative ligand complexes; and

detecting binding of the TCR-displaying phage to the putative ligand complexes.

TCR ligands suitable for identification by the above method include, but are not limited to, peptide-MHC complexes.

*Screening Use*

The TCR-displaying phage particles of the present invention are capable of utilisation in screening methods designed to identify modulators, including inhibitors, of the TCR-mediated cellular immune synapse.

As is know to those skilled in the art there are a number of assay formats that provide a suitable basis for protein-protein interaction screens of this type.

Amplified Luminescent Proximity Homogeneous Assay systems such as the AlphaScreen™, rely on the use of "Donor" and "Acceptor" beads that are coated with a layer of hydrogel to which receptor and ligand proteins can be attached. The interaction between these receptor and ligand molecules brings the beads into proximity. When these beads are subject to laser light a photosensitizer in the "Donor" bead converts ambient oxygen to a more excited singlet state. The singlet state oxygen molecules diffuse across to react with a chemiluminescer in the "Acceptor" bead that further activates fluorophores contained within the same bead. The fluorophores subsequently emit light at 520-620 nm, this signals that the receptor-ligand interaction has occurred. The presence of an inhibitor of the receptor-ligand interaction causes this signal to be diminished.

Surface Plasmon Resonance (SPR) is an interfacial optical assay, in which one binding partner (normally the receptor) is immobilised on a 'chip' (the sensor surface) and the binding of the other binding partner (normally the ligand), which is soluble and is caused to flow over the chip, is detected. The binding of the ligand results in an increase in concentration of protein near to the chip surface which causes a change in the refractive index in that region. The surface of the chip is comprised such that the change in refractive index may be detected by surface plasmon resonance, an optical

phenomenon whereby light at a certain angle of incidence on a thin metal film produces a reflected beam of reduced intensity due to the resonant excitation of waves of oscillating surface charge density (surface plasmons). The resonance is very sensitive to changes in the refractive index on the far side of the metal film, and it is this signal which is used to detect binding between the immobilised and soluble proteins. Systems which allow convenient use of SPR detection of molecular interactions, and data analysis, are commercially available. Examples are the Iasys™ machines (Fisons) and the Biacore™ machines.

Other interfacial optical assays include total internal reflectance fluorescence (TIRF), resonant mirror (RM) and optical grating coupler sensor (GCS), and are discussed in more detail in Woodbury and Venton (*J. Chromatog. B.* **725** 113-137 (1999)).

The scintillation proximity assay (SPA) has been used to screen compound libraries for inhibitors of the low affinity interaction between CD28 and B7 ($K_d$ probably in the region of 4 μM (Van der Merwe *et al* J. Exp. Med. **185**:393-403 (1997), Jenh *et al,* Anal Biochem **165**(2) 287-93 (1998)). SPA is a radioactive assay making use of beta particle emission from certain radioactive isotopes which transfers energy to a scintillant immobilised on the indicator surface. The short range of the beta particles in solution ensures that scintillation only occurs when the beta particles are emitted in close proximity to the scintillant. When applied for the detection of protein-protein interactions, one interaction partner is labelled with the radioisotope, while the other is either bound to beads containing scintillant or coated on a surface together with scintillant. If the assay can be set up optimally, the radioisotope will be brought close enough to the scintillant for photon emission to be activated only when binding between the two proteins occurs.

A further aspect of the invention is a method of identifying an inhibitor of the interaction between a TCR-displaying phage particle of the invention, and a TCR-binding ligand comprising contacting the TCR-displaying phage particle with a TCR-binding ligand, in the presence of and in the absence of a test compound, and determining whether the presence of the test compound reduces binding of the TCR-

displaying phage particle to the TCR-binding ligand, such reduction being taken as identifying an inhibitor.

A final aspect of the invention is a method of identifying a potential inhibitor of the interaction between an TCR-displaying phage particle of the invention, and a TCR-binding ligand, for example a MHC-peptide complex, comprising contacting the TCR-displaying phage particle or TCR-binding ligand partner with a test compound and determining whether the test compound binds to the TCR-displaying phage particle and/or the TCR-binding ligand, such binding being taken as identifying a potential inhibitor. This aspect of the invention may find particular utility in interfacial optical assays such as those carried out using the BIAcore™ system.

Preferred features of each aspect of the invention are as for each of the other aspects *mutatis mutandis*. The prior art documents mentioned herein are incorporated to the fullest extent permitted by law.

**Examples**

The invention is further described in the following examples, which do not limit the scope of the invention in any way.

~~Reference is made in the following to the accompanying drawings in which:~~

Figures 1a and 1b show respectively the nucleic acid sequences of the α and β chains of a soluble A6 TCR, mutated so as to introduce a cysteine codon. The shading indicates the introduced cysteine codons;

Figure 2a shows the A6 TCR α chain extracellular amino acid sequence, including the $T_{48} \rightarrow C$ mutation (underlined) used to produce the novel disulphide inter-chain bond, and Figure 2b shows the A6 TCR β chain extracellular amino acid sequence, including

the $S_{57} \rightarrow C$ mutation (underlined) used to produce the novel disulphide inter-chain bond;

Figure 3 Outlines the cloning of TCR α and β chains into phagmid vectors. The diagram describes a phage display vector. RSB is the ribosome-binding site. S1 or S2 are signal peptides for secretion of proteins into periplasm of *E. coli*. The * indicates translation stop codon. Either of the TCR α chain or β chain can be fused to phage coat protein, however in this diagram only TCR β chain is fused to phage coat protein.

Figure 4 details the DNA sequence of phagmid pEX746:A6.

Figure 5 expression of phage particles fusions of bacterial coat protein and dTCR in E. coli. Fusion proteins of dTCR::gIII are detected using western blotting. Phage particles are prepared from E. cloi XL-1-Blue and concentrated with PEG/NaCl. The samples are loaded in reducing or non-reducing sample buffers. Lane 1 is the sample of clone 1 containing correct sequence, and lane 2 is the sample of clone 2 containing a deletion in the α-chain encoding gene. The dsTCR::gIII fusion protein was detected at 125kDa.

Fig 6 illustrates ELISA detection of pMHC peptide complex binding activity of dTCR displayed on phage. Clone 1 binds specifically to HLA A2/Tax complex. Clone 2 can not bind to any pMHC, as no dTCR is attached to the phage particles.

*Example 1 – Design of primers and mutagenesis of A6 Tax TCR α and β chains to introduce the cysteine residues required for the formation of a novel inter-chain disulphide bond*

For mutating A6 Tax threonine 48 of exon 1 in TRAC*01 to cysteine, the following primers were designed (mutation shown in lower case):

5'-C ACA GAC AAA tgT GTG CTA GAC AT
5'-AT GTC TAG CAC Aca TTT GTC TGT G

For mutating A6 Tax serine 57 of exon 1 in both TRBC1*01 and TRBC2*01 to cysteine, the following primers were designed (mutation shown in lower case):

5'-C AGT GGG GTC tGC ACA GAC CC
5'-GG GTC TGT GCa GAC CCC ACT G

*PCR mutagenesis:*

Expression plasmids containing the genes for the A6 Tax TCR α or β chain were mutated using the α-chain primers or the β-chain primers respectively, as follows. 100 ng of plasmid was mixed with 5 µl 10 mM dNTP, 25 µl 10xPfu-buffer (Stratagene), 10 units Pfu polymerase (Stratagene) and the final volume was adjusted to 240 µl with $H_2O$. 48 µl of this mix was supplemented with primers diluted to give a final concentration of 0.2 µM in 50 µl final reaction volume. After an initial denaturation step of 30 seconds at 95°C, the reaction mixture was subjected to 15 rounds of denaturation (95°C, 30 sec.), annealing (55°C, 60 sec.), and elongation (73°C, 8 min.) in a Hybaid PCR express PCR machine. The product was then digested for 5 hours at 37°C with 10 units of DpnI restriction enzyme (New England Biolabs). 10 µl of the digested reaction was transformed into competent XL1-Blue bacteria and grown for 18 hours at 37°C. A single colony was picked and grown over night in 5 ml TYP + ampicillin (16 g/l Bacto-Tryptone, 16 g/l Yeast Extract, 5 g/l NaCl, 2.5 g/l

$K_2HPO_4$, 100 mg/l Ampicillin). Plasmid DNA was purified on a Qiagen mini-prep column according to the manufacturer's instructions and the sequence was verified by automated sequencing at the sequencing facility of Department of Biochemistry, Oxford University. The respective mutated nucleic acid and amino acid sequences are shown in Figures 1a and 2a for the α chain and Figures 1b and 2b for the β chain.

*Example 2 - Construction of phage display vectors and cloning of α and β chains into the phagemid vectors.*

In order to display dTCR on filamentous phage particles, phagemid vectors were constructed for expression of fusion proteins comprising dTCR with a phage coat protein. These vectors contain a pUC19 origin, an M13 origin, a bla (Ampicillin resistant) gene, Lac promoter/operator and a CAP-binding site. The design of these vectors is outlined in Figure 3., which describes vectors encoding for both dTCR β chain-gp3 or dTCR β chain-gp8 fusion proteins in addition to a soluble TCR α chain. The expression vectors containing the DNA sequences of the mutated A6 TCR α and β chains incorporating the additional cysteine residues required for the formation of a novel disulphide prepared in Example 1 and as shown in figures 1a and 1b were used as the source of the A6 TCR α and β chains for the production of a phagemid encoding this TCR. The complete DNA sequence of the phagemid construct utilised is given in Figure 4.

The molecular cloning methods for constructing the vectors are described in "Molecular cloning: A laboratory manual, by J. Sambrook and D. W. Russell". Primers listed in table-1 are used for construction of the vectors. A example of the PCR programme is 1 cycle of 94°C for 2 minutes, followed by 25 cycles of 94°C for 5 seconds, 53°C for 5 seconds and 72°C for 90 seconds, followed by 1 cycles of 72°C for 10 minutes, and then hold at 4°C. The hifidelity Taq DNA polymerase is purchased from Roche.

Table 1. Primers used for construction of dTCR phage display vectors

| Primer name | Sequence 5' to 3' | Usage |
|---|---|---|
| YOL1 | TAATAATACGTATAATAATATTCTATTTCAAG GACACACTC | pEX746 construction, SnaB1/ clone SDII |
| YOL2 | CAATCCAGCGGCTGCCGTAGGCAATAGGTATT TCATTATGACTGTCTCCTTGAAATAG | pEX746 construction, clone s1 |
| YOL3 | CtaCGGCAGCCGCTGGATTGTTATTACTCGCG GCCCAGCCGGCCATGGCccag GTTCTGCTCCACTTCCTTCTGGGCCATGGCCG | Clone S1 |
| YOL4 | GCTGGGCCG | Fuse S1 and A6 Vα |
| YOL5 | CAGAAGGAAGTGGAGCAGAAC CTTCTTAAAGAATTCTTAATTAACCTAGGTTA | Clone A6 Vα |
| YOL6 | TTAGGAACTTTCTGGGCTGGGGAAG GTTAATTAAGAATTCTTTAAGAAGGAGATATA | Clone A6 Cα |
| YOL7 | CATATGAAAAAATTATTATTCGCAATTC CGCGCTGTGAGAATAGAAAGGAACAACTAAAG | Clone SDIII |
| YOL8 | GAATTGCGAATAATAATTTTTTCATATG CTTTCTATTCTCACAGCGCGCAGGCTGGTGTC | Clone S2 |
| YOL9 | ACTCAGAC ATGATGTCTAGATGCGGCCGCGTCTGCTCTAC | Clone S2 and A6 Vβ |
| YOL10 | CCCAGGCCTC GCATCTAGACATCATCACCATCATCACTAGAC | Clone A6 Cβ Clone $(His)_6$ tag and amber |
| YOL11 | TGTTGAAAGTTGTTTAGCAAAAC CTAGAGGGTACCTTATTAAGACTCCTTATTAC | codon and fuse to gIII |
| YOL12 | GCAGTATG | Clone gIII |

*Example 3 - Expression of fusions of bacterial coat protein and dTCR in E. coli.*

In order to validate the constructs made in Example 2, phage particles displaying dTCR are prepared using methods described previously for the generation of phage particles displaying antibody scFvs (Li *et al*, 2000, Journal of Immunological Methods **236:** 133-146) with the following modifications. E. coli XL-1-Blue cells containing pEX746:A6 phagemid were used to inoculate 5 ml of LBatg (Lennox L broth containing 100μg/ml of ampicillin, 12.5 μg/ml tetracycline and 2% glucose), and then

the culture was incubated with shaking at 37°C overnight (16 hours). 50 µl of the overnight culture was used to inoculate 5 ml of TYPatg (TYP is 16g/l of peptone, 16g/l of yeast extract, 5g/l of NaCl and 2.5g/l of $K_2HPO_4$), and then the culture was incubated with shaking at 37°C until $OD_{600nm}$ = 0.8. Helper phage M13 K07 was added to the culture to the final concentration of 5 X $10^9$ pfu/ml. The culture was then incubated at 37°C stationary for thirty minutes and then with shaking at 200 rpm for further 30 minutes. The medium of above culture was then changed to TYPak (TYP containing 100µg/ml of ampicillin, 25 µg/ml of kanamycin), the culture was then incubated at 25°C with shaking at 250 rpm for 36 to 48 hours. The culture was then centrifuged at 4°C for 30 minutes at 4000 rpm. The supernatant was filtrated through a 0.45 µm syringe filter and stored at 4°C for further concentration or analysis.

The fusion protein of filamentous coat protein and dsTCR was detected by western blotting. Approximately $10^{11}$ cfu phage particles were loaded on each lane of an SDS-PAGE gel in both reducing and non-reducing loading buffer. Separated proteins were probed primary with an anti-M13 gIII mAb, followed by a second antibody conjugated with Horseradish Peroxidase (HRP). The HRP activity was then detected with Opti-4CN substrate kit from Bio-Rad (Figure 5). The data indicated that disulphide-bonded TCR of clone 1 is fused with filamentous phage coat protein, gIII protein.

*Example 4 - Detection of functional dTCR on filamentous phage particles*

The presence of functional (cognate peptide-MHC binding) dTCR displayed on the phage particles was detected using a phage ELISA method.

*TCR-Phage ELISA*

Binding of the TCR-dispalying phage particles to immobilised peptide-MHC in ELISA is detected with primary rabbit anti-fd antisera (Sigma) followed by alkaline phosphatase (AP) conjugated anti-Rabbit mAb (Sigma). Non specific protein binding sites in the plates can be blocked with 2% MPBS or 3% BSA-PBS

*Materials and reagents*

1. Coating buffer, PBS
2. PBS: 138mM NaCl, 2.7mM KCl, 10mM $Na_2HPO_4$, 2mM $KH_2PO_4$
3. MPBS , 3% marvel-PBS
4. PBS-Tween: PBS, 0.1% Tween-20
5. Substrate solution, Sigma FAST pNPP, Cat# N2770

*Method*

1. Rinse NeutrAvidin coated wells twice with PBS.
2. Add 25µl of biotin-HLA A2 Tax or biotin-HLA A2 NYESO in PBS at concentration of 10 µg/ml, and incubate at room temperature for 30 to 60 min.
3. Rinse the wells twice with PBS
4. Add 300 µl of 3% Marvel-PBS, and incubate at room temperature for 1hr. Mix the TCR-phage suspension with 1 volume of 3% Marvel-PBS and incubate at room temperature.
5. Rinse the wells twice with PBS
6. Add 25 µl of the mixture of phage-TCR/Marvel-PBS, incubate on ice for 1hr
7. Rinse the wells three times with ice-cold PBStween, and three times with ice-cold PBS.
8. Add 25 µl of ice cold rabbit anti-fd antibody diluted 1:1000 in Marvel-PBS, and incubate on ice for 1hr
9. Rinse the wells three times with ice-cold PBStween, and three times with ice-cold PBS.
10. Add 25 µl of ice cold anti-rabbit mAb-Ap conjugate diluted 1:50,000 in Marvel-PBS, and incubate on ice for 1hr
11. Rinse the wells three times with ice-cold PBStween, and three times with ice-cold PBS.
12. Add 150 µl of Alkaline phosphatase yellow to each well and read the signal at 405nm

The results presented in Figure 6 indicate clone 1 produced a phage particle displaying a dTCR which can bind specifically to its cognate pMHC.

Claims:

1. A phage particle displaying on its surface a dimeric T-cell receptor (dTCR) polypeptide pair, or a single chain T-cell receptor (scTCR) polypeptide, wherein

the dTCR polypeptide pair is constituted by TCR amino acid sequences corresponding to extracellular constant and variable region sequences present in native TCR chains, and the scTCR is constituted by TCR amino acid sequences corresponding to extracellular constant and variable region sequences present in native TCR chains and a linker sequence, the latter linking a variable region sequence corresponding to that of one chain of a native TCR to a constant region sequence corresponding to a constant region sequence of another native TCR chain;

the variable region sequences of the dTCR polypeptide pair or scTCR polypeptide are mutually orientated substantially as in native TCRs; and

in the case of the scTCR polypeptide a disulfide bond which has no equivalent in native T cell receptors links residues of the polypeptide.

2. A phage particle as claimed in claim 1 wherein the C-terminus of one member of the dTCR polypeptide pair, or the C-terminus of the scTCR polypeptide, is linked by a peptide bond to a surface exposed residue of the phage.

3. A phage particle as claimed in claim 1 wherein the C-terminus of one member of the dTCR polypeptide pair, or the C-terminus of the scTCR polypeptide, is linked by a disulfide bond to a surface exposed cysteine residue of the phage.

4. A phage particle as claimed in any of claims 1 to 3 wherein a scTCR polypeptide is displayed which has

a first segment constituted by an amino acid sequence corresponding to a TCR α or δ chain variable region sequence fused to the N terminus of an amino acid sequence corresponding to a TCR α chain constant region extracellular sequence,

a second segment constituted by an amino acid sequence corresponding to a TCR β or γ chain variable region fused to the N terminus of an amino acid sequence corresponding to TCR β chain constant region extracellular sequence,

a linker sequence linking the C terminus of the first segment to the N terminus of the second segment, or vice versa, and

a disulfide bond between the first and second chains, said disulfide bond being one which has no equivalent in native αβ or γδ T cell receptors,

the length of the linker sequence and the position of the disulfide bond being such that the variable region sequences of the first and second segments are mutually orientated substantially as in native αβ or γδ T cell receptors.

5. A phage particle as claimed in claim 4 wherein the linker sequence has the formula -P-AA-P- wherein P is proline and AA represents an amino acid sequence wherein the amino acids are glycine and serine.

6. A phage particle as claimed in claim 4 or claim 5 wherein the linker sequence links the C terminus of the first segment to the N terminus of the second segment.

7. A phage particle as claimed in claim 6 wherein the linker sequence consists of from 26 to 41 amino acids.

8. A phage particle as claimed in claim 7 wherein the linker sequence consists of 29, 30, 31 or 32 amino acids.

9. A phage particle as claimed in claim 7 wherein the linker sequence consists of 33, 34, 35 or 36 amino acids.

10. A phage particle as claimed in claim 7 wherein the linker sequence has the formula -PGGG-$(SGGGG)_5$-P- wherein P is proline, G is glycine and S is serine.

11. A phage particle as claimed in claim 7 wherein the linker sequence has the formula -PGGG-$(SGGGG)_6$-P- wherein P is proline, G is glycine and S is serine.

12. A phage particle as claimed in any of claims 1 to 3 wherein a dTCR polypeptide pair is displayed which is constituted by

a first polypeptide wherein a sequence corresponding to a TCR α or δ chain variable region sequence is fused to the N terminus of a sequence corresponding to a TCR α chain constant region extracellular sequence, and

a second polypeptide wherein a sequence corresponding to a TCR β or γ chain variable region sequence fused to the N terminus a sequence corresponding to a TCR β chain constant region extracellular sequence,

the first and second polypeptides being linked by a disulfide bond which has no equivalent in native αβ or γδ T cell receptors.

13. A phage particle as claimed in any preceding claim wherein the displayed dTCR polypeptide pair or scTCR polypeptide have amino acid sequences corresponding to αβ TCR extracellular constant and variable region sequences.

14. A phage particle as claimed in any of claims 1 to 12 wherein the displayed dTCR polypeptide pair or scTCR polypeptide have amino acid sequences

corresponding to extracellular αβ TCR constant region sequences and γδ TCR variable region sequences.

15. A phage particle as claimed in any preceding claim wherein the displayed dTCR polypeptide pair or scTCR polypeptide have amino acid sequences corresponding to non-human extracellular αβ TCR constant region sequences and human TCR variable region sequences.

16. A phage particle as claimed in any of claims 1 to 15 wherein an amino acid sequence of one member of the displayed dTCR polypeptide pair, or an amino acid sequence of the displayed scTCR, corresponds to a native TCR extracellular constant chain Ig domain sequence.

17. A phage particle as claimed in any of claims 1 to 15 wherein the displayed dTCR polypeptide pair or displayed scTCR, includes sequences corresponding to native TCR extracellular constant chain Ig domain sequences.

18. A phage particle as claimed in claim 17 wherein a disulfide bond links amino acid residues of the said constant chain Ig domain sequences, which disulfide bond has no equivalent in native TCRs.

19. A phage particle as claimed in claim 18 wherein the said disulfide bond is between cysteine residues corresponding to amino acid residues whose β carbon atoms are less than 0.6 nm apart in native TCRs.

20. A phage particle as claimed in claim 19 wherein the said disulfide bond is between cysteine residues substituted for Thr 48 of exon 1 of TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01 or the non-human equivalent thereof.

21. A phage particle as claimed in claim 19 wherein the said disulfide bond is between cysteine residues substituted for Thr 45 of exon 1 of TRAC*01 and Ser 77 of exon 1 of TRBC1*01 or TRBC2*01 or the non-human equivalent thereof.

22. A phage particle as claimed in claim 19 wherein the said disulfide bond is between cysteine residues substituted for Tyr 10 of exon 1 of TRAC*01 and Ser 17 of exon 1 of TRBC1*01 or TRBC2*01 or the non-human equivalent thereof.

23. A phage particle as claimed in claim 19 wherein the said disulfide bond is between cysteine residues substituted for Thr 45 of exon 1 of TRAC*01 and Asp 59 of exon 1 of TRBC1*01 or TRBC2*01 or the non-human equivalent thereof.

24. A phage particle as claimed in claim 19 wherein the said disulfide bond is between cysteine residues substituted for Ser 15 of exon 1 of TRAC*01 and Glu 15 of exon 1 of TRBC1*01 or TRBC2*01 or the non-human equivalent thereof.

25. A phage particle as claimed in any of claims 17 to 24 wherein the sequences corresponding to native TCR extracellular constant chain Ig domain sequences are truncated at their C-termini relative to said native sequences such that the cysteine residues which form the native interchain disulphide bond are excluded.

26. A phage particle as claimed in any of claims 17 to 24 wherein in the sequences ~~corresponding to native TCR extracellular constant chain Ig domain sequences the~~ cysteine residues which form the native interchain disulphide bond are substituted by non-cysteine residues.

27. A phage particle as claimed in claim 26 wherein the cysteine residues which form the native interchain disulfide bond are substituted by serine or alanine.

28. A phage particle as claimed in any of claims 1 to 27 wherein in the displayed dTCR or scTCR there is no unpaired cysteine residue corresponding an unpaired cysteine residue present in a native TCR.

29. A phage particle as claimed in any of claims 17 to 28 wherein the sequences corresponding to native TCR extracellular constant chain Ig domain sequences are truncated N-terminal to residues corresponding to those which form the non-native interchain disulphide bond.

30. A phage particle as claimed in any preceding claim wherein the C-terminus of one member of the dTCR polypeptide pair, or the C-terminus of the scTCR polypeptide, is linked by a disulfide bond to a surface exposed cysteine residue of the phage and wherein said surface exposed cysteine residue is at the N-terminus of the gene product of bacteriophage gene III or gene VIII.

31. A phagemid or phage genome genetic sequence which encodes a dTCR polypeptide pair, or a scTCR polypeptide, having the structural features defined in any of claims 1 to 30.

32. A composition comprising a phagemid genetic sequence as claimed in claim 31 and a helper phage.

33. A composition as claimed in claim 32 wherein non-TCR genetic sequences are derived from a filamentous phage

34. A composition as claimed in claim 33 wherein the helper phage is M13K07 or VCS M13 filamentous phage

35. A host bacterial cell comprising a composition as claimed in any of claims 31 to 34.

36. A method for obtaining a phage particle as claimed in any of claims 1 to 30, which method comprises incubating a host cell as claimed in claim 35 under conditions causing expression of polypeptides which self assemble to form the TCR-displaying phage and then purifying the said TCR-displaying phage.

37. A method for detecting TCR ligand complexes, which comprises:

(i) providing a TCR-displaying phage as claimed in any of claims 1 to 30;

(ii) contacting said TCR-displaying phage particle with a putative ligand complex; and

(iii) detecting binding of the said TCR-displaying phage particle to the putative ligand complexes

38. A method as claimed in claim 37 wherein the putative TCR-binding complexes are peptide-MHC complexes.

39. A method of identifying an inhibitor of the interaction between a TCR-displaying phage particle as claimed in any one of claims 1 to 30, and a TCR-binding ligand comprising contacting the TCR-displaying phage particle with a TCR-binding ligand, in the presence of and in the absence of a test compound, and determining whether the presence of the test compound reduces binding of the TCR-displaying phage particle to the TCR-binding ligand, such reduction being taken as identifying an inhibitor.

40. A method of identifying a potential inhibitor of the interaction between an TCR-displaying phage particle as claimed in any one of claims 1 to 30, and a TCR-binding ligand comprising contacting the TCR-displaying phage particle or TCR-binding ligand partner with a test compound and determining whether the test compound binds to the TCR-displaying phage particle and/or the TCR-binding ligand, such binding being taken as identifying a potential inhibitor.

**Figure 1a**

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**Figure 1b**

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**Figure 2a**

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PESS*

**Figure 2b**

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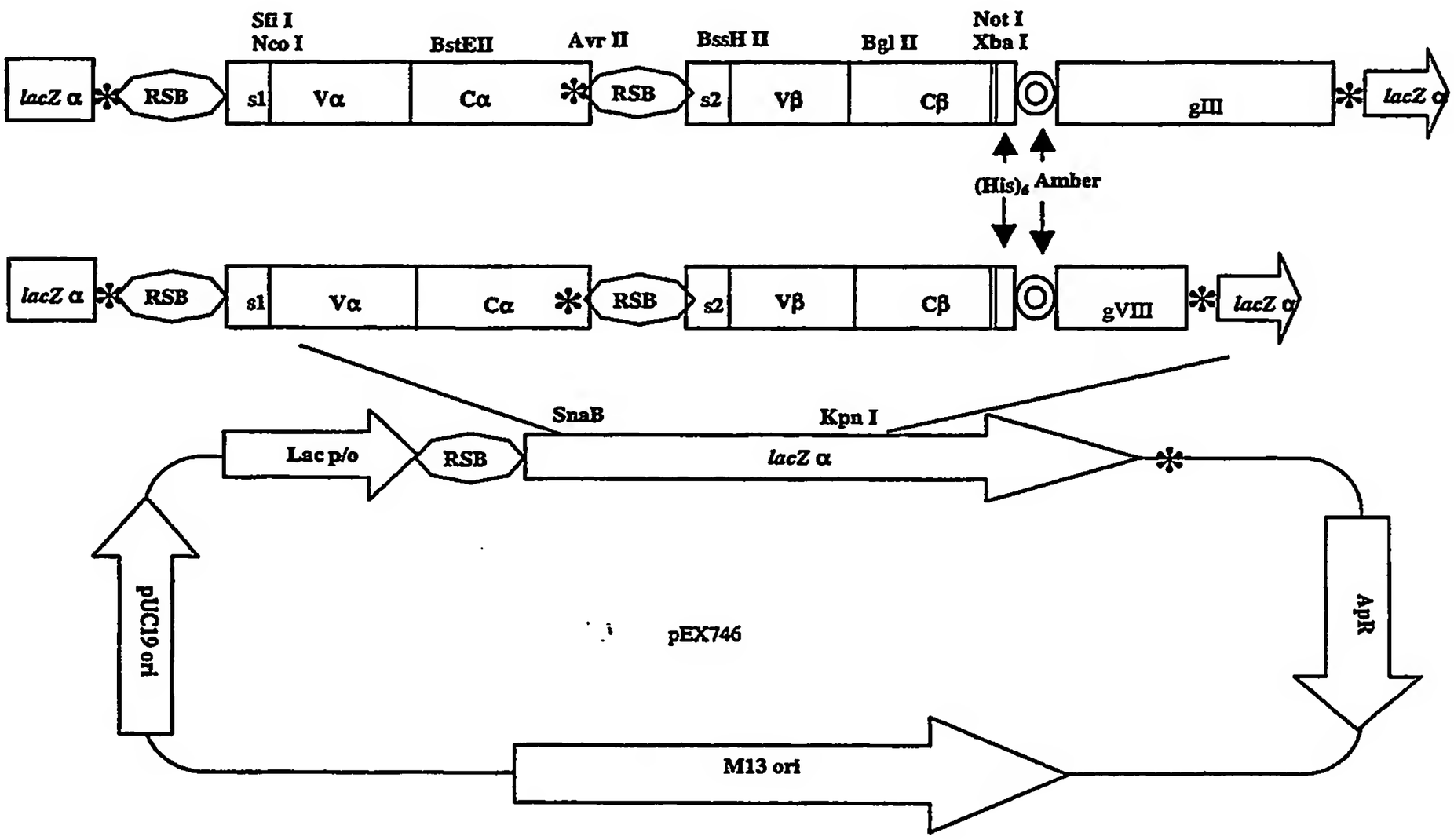
**Figure 3**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RSB
s2
Vβ
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gIII
lacZ α
(His)6
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lacZ α
RSB
s1
Vα
Cα
RSB
s2
Vβ
Cβ
gVIII
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Lac p/o
RSB
lacZ α
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pEX746
ApR
M13 ori

# Figure 4

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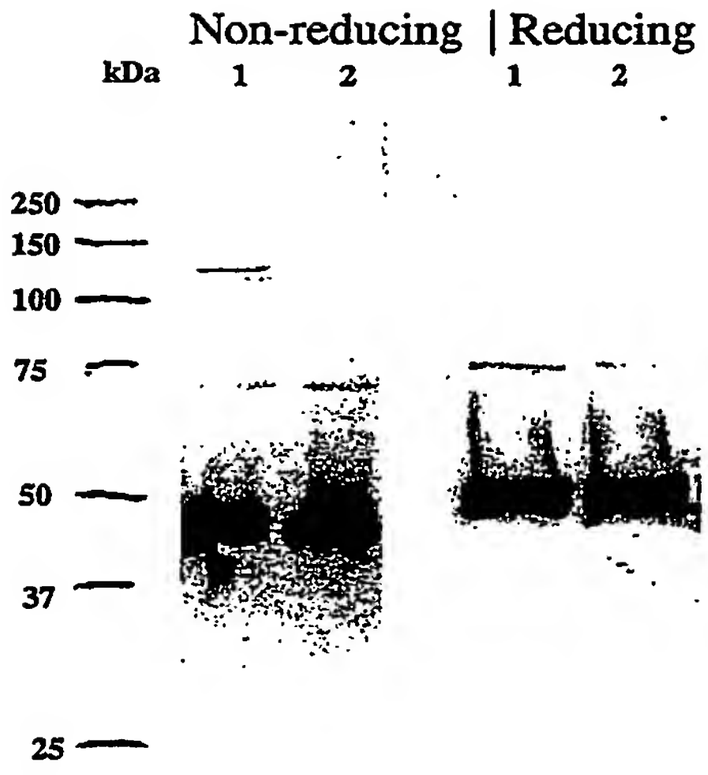
**Figure 5**



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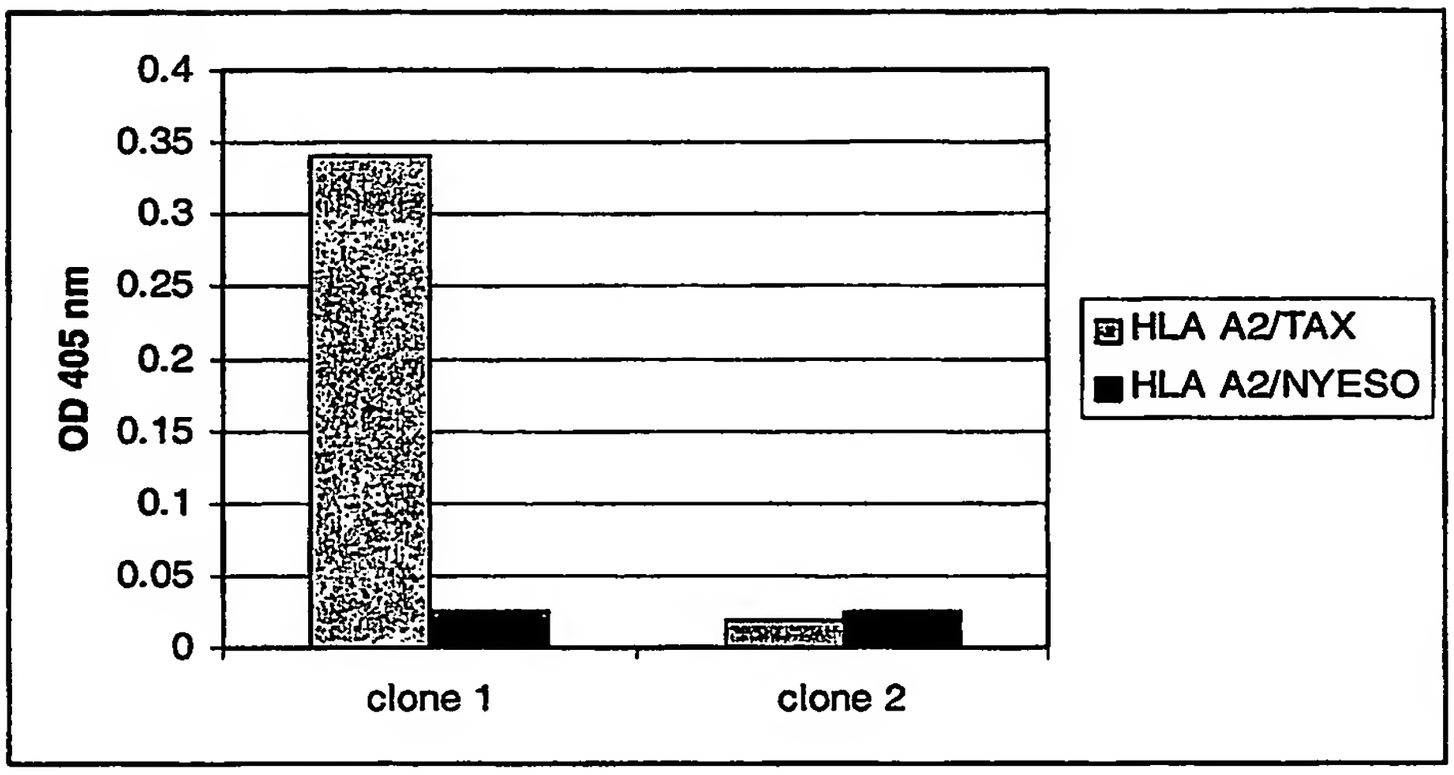
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